Protective Effect of Ethanolic Extract of *Spirulina platensis* on Reproductive Characteristic and Biochemical Profile in Female Guinea Pig (*Cavia porcellus*) Exposed to Lead Acetate

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**Authors’ contributions**

This work was carried out in collaboration among all authors. Authors DNS and NF designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors TH, MNJL and VBN managed the analyses of the study. Author TJ managed the literature searches. All authors read and approved the final manuscript.

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**ABSTRACT**

The effect of ethanolic extract of *Spirulina platensis* (EESP) on reproductive function of female guinea pigs exposed to oxidative stress induced by lead acetate was explored. 60 mature female guinea pigs were used. They were divided into 6 groups of 10 guinea pigs each. For 3 months, pigs in Groups 1 were orally given distilled water daily; Group 2 were treated with lead; Group 3: received lead acetate and Vitamin C while Groups 4, 5 and 6 were given lead acetate with 50, 100 and 200 mg of EESP/kg.bw respectively by gavage. At the end of trial (90 days), 6 female pigs per group were sacrificed and some reproductive characteristics, oxidative stress and hepatic toxicity markers were evaluated. Results revealed that, exposure to Lead acetate induced a significant...
1. INTRODUCTION

In recent decades, there has been major interest in the potential negative effects of environmental hazardous chemicals on reproductive function [1]. Lead is one of the most expanded contaminants amongst the myriad of xenobiotic [2] which might be a major risk factor considering the hormonal disruption [3] associated with the reduction of sex organs weight and decrease of fertility [4]. Lead is an environmental pollutant that has been detected in many part of world and biological systems [5]. Lead is a major industrial byproduct and can be present in water pipes, insecticides, lining of equipment where corrosion resistance are required, in construction, bullets of gun, x-ray and atomic radiation protection. Lead is toxic for human and animal [6-8]. It is considered as one of the most hazardous and environmental pollutants that affect all biological systems through air exposure, food source and water [9]. The negative effects of lead on the organism in general and particularly in reproductive function have been widely studied [10-11]. Heavy metals such as lead generally show their harmful effects through reactive oxygen species (ROS) production or inhibition of antioxidant enzyme activities [12,13]. In fact, Upasani et al. [14] reported that lead toxicity is associated with increased lipid peroxidation. Also lead induces the production of reactive oxygen species (ROS) causing oxidative damage in various tissues [15]. Moreover, exposure to lead causes increased lipid peroxidation in membranes of many tissues, where thiobarbituric acid reactive substances and hydroperoxides are signs of oxidative damage [4] [16]. Intake of lead increase the use of glutathione (GSH) and protein binding sulhydryl groups and thus improve the levels of free radicals, such as hydrogen peroxide, hydroxide, and superoxide anions [17]. The liver and kidney due to their detoxification function are vulnerable to oxidative toxicity [9,18]. The increase of serum hepatic markers levels, such as aspartate aminotransferase (AST) and alanine transaminase (ALT) following lead contamination suggests the damaging effects of heavy metal like lead on the liver [19-20]. Al-Harby [21] and Ait Hamadouche et al. [22] reported that lead toxicity comes from the production of free radicals and the induction of cell necrosis and apoptosis which can result in reproductive damage.

Several measures to neutralize the oxidative damage in animal organism is the use of antioxidants. Nowadays, the use of natural herbs to reduce oxidative damage has increased worldwide. This is because the antioxidant compounds present in these herbs are possibly effective against lead toxicity [23,17]. One of these herbal is Spirulina platensis.

Spirulina platensis is a unicellular cyanobacterium belonging to cyanophyceae class, oscillatoriaceae family. It is characterized by spiral chains of cells enclosed in a thin sheath. It contains many potent components, natural antioxidant molecules and free radical scavenging agents [24]. Spirulina is a micro vegetable widely used as an animal feed supplement [25]. It is nontoxic, available, and provide significant protection against environmental contaminants, drugs and other chemicals induced toxic offensive of [26]. It has been shown to be a wealth source of vitamins especially vitamin E, proteins, minerals, essential fatty acids and phytoestrogen compounds [27], powerful antioxidant pigments such as phycocianin and carotenoids [28,24]. The present study investigated the protective ability of ethanolic extract of Spirulina platensis (EESP) against lead induced damages on reproductive characteristics and blood biochemical profiles in guinea pigs.

Keywords: Spirulina platensis; lead acetate; oxidative stress; female guinea pigs.
2. MATERIALS AND METHODS

2.1 Animals

Sixty (60) female adult guinea pigs aged 3-4 months, with a mean body weight of 350 ± 5.3 g were obtained with their usual diets from the teaching and research farm of the university of Dschang. These animals were maintained on the normal diets at ambient temperature, 12/12-hr light/dark cycle, ventilation, and hygienic conditions.

2.1.1 Chemicals

Lead acetate was obtained from commercial sources, Trust chemical laboratories, United Kingdom; P.NO AIP/ 20140112UN / 2915.2990. Vitamine C was also obtained from commercial sources; Shalina, Nariman point, Mumbai, Inde. A/Em/At: Plot No. E-2, M.I.D.C. Jejuri; Tal: Purandar. Dist: Pune, Maharashtra, India. www.shalina.com. Shalina, Nariman point, Mumbai, Inde. A/Em/At: Plot No. E-2, M.I.D.C. Jejuri; Tal: Purandar. Dist: Pune, Maharashtra, India. www.shalina.com

2.2 Experimental Design

The animals were divided in 6 groups of 10 animals as follows:

**Group 1:** Negative control received by gavage distilled water.

**Groups 2:** Positive control received by gavage 12 mg of lead acetate/kg/bw.

**Groups 3:** Co-treated with 12 mg of lead acetate/kg/bw and 100 mg/kg/bw of vitamin C.

**Groups 4:** Co-treated with 12 mg of lead acetate/kg/bw and 50 mg of ethanolic extract of *Spirulina platensis* (EESP) /kg/bw.

**Groups 5:** Co-received 12 mg of lead acetate/kg/bw along and100 mg of EESP/kg/bw.

**Groups 6:** Co-exposed to 12 mg of lead acetate/kg/bw and 200 mg of EESP/kg/bw.

The experiment lasted 90 days. After one month of the treatment, an untreated male was introduced into each cage for a period of one week. On the 90th day, 6 guinea pigs per group were killed by cervical dislocation under light ether anesthesia. Blood samples were collected from ventral aorta and stored at room temperature for 6 hours after which serum was collected and kept at -20°C for biochemical analysis.

Liver fragment of each pig was homogenized in a known volume of cold 0.9% NaCl followed by a centrifugation (3000 rpm, 30 min), and the resultant supernatants were stored at −20°C for assessment of superoxide dismutase (SOD), catalase (CAT), and peroxidase glutathione (GPx) activities, malondialdehyde concentration.

2.3 Evaluation of the Fertility

After slaughter, the number of pregnant guinea pigs and fetuses were recorded. The sexual organs (Gravid uterine, Empty uterine, Ovaries) and embryo weights were also registered. The fertility rate was calculated by dividing the number of fertile guinea pigs over the total number of guinea pigs killed. The individual fetal weight was obtained by dividing the total Fetal weight over the number of fetuses per group. The fetal mortality rate was obtained by dividing the number of fetus death over the total number of fetuses recorded.

2.4 Biochemical Analysis

The levels of total proteins, creatinine, urea, total cholesterol, Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the serum were determined using CHRONOLAB kit following the manufacturer's protocol. Serum hormones (Follicle stimulating hormones (FSH), luteinizing hormone (LH) and progesterone) were determined as describe by appropriate kit (ELISA AccuDiagTM, Diagnostic Automation Inc). The concentration of MDA and the activities of SOD, CAT and GPx were assessed in the liver tissues according to the methods described by [29,30,31,32] respectively, using a spectrophotometer (GENESYS 20.0).

2.5 Evaluation of Sex Organs Weight

The gravid uterus, empty uterus, and ovary weights were measured with the aid of an electronic scale of 160 g capacity and 10⁻³ g precision.

2.6 Tissue Preparation and Histopathology

The ovaries of each female were fixed in Bouin's fluid for 1 week, embedded in paraffin,
3. RESULTS

3.1 Body Weight and Relative Weight of Reproductive Organs

The co-exposition of female guinea pig to lead acetate and *Spirulina platensis* did not significantly (p > 0.05) affect feed intake. The final body weight, the body weight gain and feed intake decreased significantly (p < 0.05) with lead acetate and increased in a dose-dependent manner with the co-administration of *Spirulina platensis* (Table 1).

3.2 Effect of EESP on Fertility, Sex Organ Weight and Embryo Characteristics of Female Guinea Pig Exposed to Lead Acetate

The fetal weight, viability and fertility indices decreased significantly (p < 0.05) in lead treated females compared to control (T0). However, the co-treatment of lead with EESP increased significantly (p < 0.05) the levels of these characteristics compared to positive control (T+) group (Table 2). The oral co-administration of lead acetate and *Spirulina platensis* to female guinea pig in 90 consecutive days did not significantly (p > 0.05) the number of fetus per female, gravid uterine weight, empty uterine weight and ovaries weight.

3.3 Protective Effects of EESP on Reproductive Serum Hormone in Pregnant Guinea Pig Exposed to Lead Acetate

The serum concentrations of FSH, LH increased while the level of progesterone declined significantly (p < 0.05) in females given only lead acetate (T-) compared to negative control (T0) and vit C treated group (Table 3). Nevertheless, co-exposition of lead and EESP to these animals significantly augmented (p < 0.05) the serum level of progesterone compared to lead acetate group (T-). The FSH level in Spirulina animal treated group was comparable to that of negative control group.

3.4 Oxidative Stress Biomarkers

The MDA concentration increased significantly (p < 0.05) while the SOD, CAT and GPx activities decreased in lead-exposed females (T-) compared to the control ones (Table 4). Co-administration of lead and EESP significantly (p < 0.05) reduced the MDA level and increased the activities of SOD, CAT and GPx.

3.5 Toxicity Biomarkers

The administration of lead acetate (T-) alone significantly (p < 0.05) increased the serum levels of ALT, AST, creatinine and urea, while total protein and cholesterol decreased significantly (p < 0.05) compared to the control (T0) (Table 5). The co-administration of lead and EESP significantly (p < 0.05) decreased ALT, AST, creatinine and urea concentrations but increased total proteins and cholesterol levels.

3.6 Histological Sections

The histology of the ovaries of treated and control group of guinea pigs are illustrated in Fig. 1. Normal structure of ovary was noticed in control group (T0). The histological section of lead acetate-treated guinea pigs (T-) showed lesions, the degeneration in the ovarian cortex and vacuolations in the stroma cells. The administration of ethanolic extract of *Spirulina platensis* attenuated ovary damages induced by lead acetate compared to those receiving the reference antioxidant (vitamin C). The dose 200 mg EESP/kg.bw showed complete restoration of the ovary’s structure.

4. DISCUSSION

The reproductive toxicity of Lead acetate in female guinea pig was characterized by a low fertility index, a reducing fetal weight, viability, ovaries and uterus weight. These results agree with those of Rus and Checiu [34] and Al-Hiyasat [35] who reported reduction in fertility indices, a decrease of fetal weight and viability, ovaries and uterus weight after chronic exposure of male rats to Cadmium and sodium fluoride respectively. The fetal mortality and weight reduction are important indicators of growth retardation of fetuses *in utero* [36]. Treatment of female guinea
### Table 1. Effect of EESP on body weight, body weight gain and feed intake in female guinea pig exposed to lead acetate

<table>
<thead>
<tr>
<th>Characteristics of reproduction</th>
<th>Controls</th>
<th>Doses of spirulina (mg/kg.bw)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(To) (n=6)</td>
<td>Pb (T-) (n=6)</td>
<td>Vit C (T+) (n=6)</td>
</tr>
<tr>
<td>Initial body</td>
<td>333.71±30.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>330±72.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>331.00±69.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Final body</td>
<td>524±79.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>476.66±71.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>505.09±59.30&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Body gain</td>
<td>191.31±23.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>146.66±61.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>152.25±33.12&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Feed intake</td>
<td>1829.4±70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1818.73±13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1753.25±131.10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

n: number of animal, each value represents mean ± standard error mean. Means values for each parameter in the same row, with different superscripts (a, b) differ significantly (p ≤ 0.05). T0: control; T-: negative control 12 mg lead acetate/kg b.w; T0+: positive control 12 mg lead acetate/kg b.w with 100mg of vitamine C; T1: lead+50 mg EESP/kg b.w; T2: lead+100 mg EESP/kg b.w. T3: lead+200 mg EESP/kg b.w. EESP: ethanolic extract of Spirulina platensis

### Table 2. Effect of EESP reproduction characteristics in female guinea pig exposed to lead acetate

<table>
<thead>
<tr>
<th>Characteristics of reproduction</th>
<th>Controls</th>
<th>Doses of spirulina (mg/kg.bw)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(To) (n=6)</td>
<td>Pb (T-) (n=6)</td>
<td>Vit C (T+) (n=6)</td>
</tr>
<tr>
<td>Fertility index (%)</td>
<td>75.00±6.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.85±3.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50.00±4.77&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>No. of fetuses/fem</td>
<td>1.50±0.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.66±0.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.60±0.89&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Viability index (%)</td>
<td>100.00±0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>80.12±27.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>100.00±0.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gravid uterine weight (g)</td>
<td>30.39±29.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.10±24.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.66±24.09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Empty uterine weight (g)</td>
<td>3.62±3.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.14±1.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.48±2.47&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ovaries weight</td>
<td>0.04±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.06±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.04±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mortality index (%)</td>
<td>27.82±10.70&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>23.66±11.60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28.66±8.32&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

n: number of animal, each value represents mean ± standard error mean. Means values for each parameter in the same row, with different superscripts (a, b) differ significantly (p ≤ 0.05). T0: control; T-: negative control 12 mg lead acetate/kg b.w; T0+: positive control 12 mg lead acetate/kg b.w with 100mg of vitamine C; T1: lead+50 mg EESP/kg b.w; T2: lead+100 mg EESP/kg b.w. T3: lead+200 mg EESP/kg b.w. EESP: ethanolic extract of Spirulina platensis, No. of fetuses/fem: number of foetus per female
### Table 3. Effects of EESP on serum concentrations of FSH, LH and Progesterone in female guinea pig exposed to lead acetate

<table>
<thead>
<tr>
<th>Serum hormones</th>
<th>0(T0) (n=6)</th>
<th>Lead(T-) (n=6)</th>
<th>Vit C(T+) (n=6)</th>
<th>50(T1) (n = 6)</th>
<th>100(T2) (n = 6)</th>
<th>200(T3) (n = 6)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH (µM/min/g)</td>
<td>34.06±7.8ab</td>
<td>35.70±7.19a</td>
<td>34.73±6.48a</td>
<td>30.78±13.35b</td>
<td>31.35±10.54b</td>
<td>31.57±12.54b</td>
<td>0.05</td>
</tr>
<tr>
<td>LH (µM/min/g)</td>
<td>81.5±17.3a</td>
<td>75.18±1.1ab</td>
<td>32.17±1.7c</td>
<td>48.08±16.86abc</td>
<td>40.37±10.9bc</td>
<td>33.33±10.69c</td>
<td>0.02</td>
</tr>
<tr>
<td>Progesteron (µM)</td>
<td>28.15±6.6a</td>
<td>15.56±4.38b</td>
<td>19.88±5.69ab</td>
<td>24.68±6.45a</td>
<td>21.82±4.54ab</td>
<td>26.87±6.72a</td>
<td>0.00</td>
</tr>
</tbody>
</table>

n: number of animal, each value represents mean ± standard error mean. Means values for each parameter in the same row, with different superscripts (a, b) differ significantly (p ≤ 0.05).

### Table 4. Effects of different levels of EESP on oxidative stress biomarkers in female guinea pig exposed to lead acetate

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls</th>
<th>Doses of spirulina (mg/kg,bw)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (µM/min/g)</td>
<td>5.49±1.46a</td>
<td>3.71±1.62a</td>
<td>6.04±0.93a</td>
</tr>
<tr>
<td>CAT(µM/min/g)</td>
<td>16.51±3.2a</td>
<td>12.77±2.89b</td>
<td>15.41±3.77ab</td>
</tr>
<tr>
<td>MDA(µM)</td>
<td>0.36±0.08a</td>
<td>0.58±0.18b</td>
<td>0.48±0.19ab</td>
</tr>
<tr>
<td>GPx (µM/min/g)</td>
<td>36.84±19.54a</td>
<td>21.15±11.26b</td>
<td>50.7±19.8b</td>
</tr>
</tbody>
</table>

n: number of animal, each value represents mean ± standard error mean. Means values for each parameter in the same row, with different superscripts (a, b) differ significantly (p ≤ 0.05).

### Table 5. Effects of EESP on toxicity biomarkers in female guinea pig exposed to lead acetate

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls</th>
<th>Doses of spirulina (mg/kg,bw)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASAT(UI/L)</td>
<td>63.54±36cd</td>
<td>159.54±28.9</td>
<td>155.96±27.6a</td>
</tr>
<tr>
<td>ALAT(UI/L)</td>
<td>68.68±14b</td>
<td>119.93±57.3</td>
<td>74.93±31.4ab</td>
</tr>
<tr>
<td>Créatinin (mg/dl)</td>
<td>0.44±0.15b</td>
<td>1.20±0.36ab</td>
<td>0.57±0.25b</td>
</tr>
<tr>
<td>Urea(mg/dl)</td>
<td>35.11±1.06cd</td>
<td>51.74±8.8a</td>
<td>32.98±6.2d</td>
</tr>
<tr>
<td>Total proteins (mg/dl)</td>
<td>3.92±0.73cd</td>
<td>3.25±1.04c</td>
<td>5.10±1.36b</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>29.9±6.87a</td>
<td>6.09±1.49b</td>
<td>28.05±5.6a</td>
</tr>
</tbody>
</table>

n: number of animal, each value represents mean ± standard error mean. Means values for each parameter in the same row, with different superscripts (a, b) differ significantly (p ≤ 0.05).
Fig. 1. Histology of ovary showing lesions
T0: control; T0-: negative control 12 mg lead acetate/kg b.w; T0+: positive control 12 mg lead acetate/kg b.w with 100 mg of vitamin C; T1: lead+50 mg EESP/kg b.w; T2: lead+100 mg EESP/kg b.w. T3: lead+200 mg EESP/kg b.w. EESP: ethanolic extract of Spirulina platensis; O1: primary ovocyte; FP: primary follicle; NO: necrosis of ovocyte; V: vascularisation; N: necrosis.
pigs with a combination of *Spirulina platensis* and lead acetate attenuated the lead induced fertility decreased and fetal mortality. These results indicate that Spirulina might have had a beneficial effect in reducing lead toxicity. This observation might be explained by the action of antioxidant compounds such as phenols especially phycocyanin, flavonoids, xanthons, terpenoids and anthaquinons [37], present in spirulina extract. The co-administration of lead acetate and EESP induced a significant increase of the serum of progesterone. This may be as a result of the antioxidative properties of phenols and flavonoids found in the extract. It might also be as a result of the action of phytohormons present in spirulina [25].

Lead treatment also resulted in increased levels of MDA as well as pathological changes in ovarian histology. Lead acetate exerts its toxic effect through the production of reactive oxygen species (ROS) which induce tissue damages [14]. ROS causes detachment and fluidity of cell membrane structure through oxidation of lipids [23]. Therefore, the increase in MDA level suggests that oxidative stress is a major mechanism by which lead acetate exerts in animal tissues. However, results of MDA assessment showed a significant decrease in groups receiving of EESP by dose dependent manner. Therefore, it could be presumed that EESP played a protective role through its antioxidant activity by inhibiting ROS production [21]. The results also indicated significant increase of ALT, AST, urea and creatinine levels in the group that was given only lead acetate suggesting liver and kidney damages. The administration of EESP revealed significant decrease of the previous parameters. Also the activities of SOD, CAT and POD in the tissue decreased in females treated with only lead acetate, however increased in spirulina-treated females. This effect suggests that spirulina has protective effects against oxidativedomages induced by lead acetate in the liver and kidney [37]. The protective role of spirulina may be due to the presence of phycocyanin, flavonoids, Vitamin C, E [25], superoxide dismutase enzyme and selenium [37]. Spirulina is a rich beta carotene food; non-enzymatic antioxidants such as carotenoids [38] play an important role in the cellular response to oxidative stress by reducing ROS at different sites to enhance antioxidant protection [39,38]. The co-exposition of animals to lead and EESP significantly decreased hepatic and kidney enzymes concentrations. This could be due to the ability of spirulina to protect tissues against alteration. According to Oguntibeju [28], antioxidant molecules have positive effects since they protect tissues against oxidation and degeneration of free radicals. Phycocyanin molecules present in spirulina significantly inhibits hydroxyl, alkoxyl, peroxyl radicals and lipid peroxidation thereby reduces peroxynitrate induced oxidative damage of DNA [39]. Phycocyanobilin (a component of phycocyanin) have essential antioxidant than α-tocopherol [27, 38]. Since lead induced reproductive toxicity involves free radical production while *Spirulina platensis* would have antioxidant and free radical scavenging properties provided the protection against reproductive damage.

5. CONCLUSION

It can be concluded that lead acetate induced oxidative stress which reduced the reproductive function in female guinea pig. However, treatment with Spirulina counteracted the action of ROS and ameliorated reproduction characteristics. Therefore, Spirulina at doses of 100 to 200 mg/kg,pc protected against lead-induced toxicity and enhanced the levels of antioxidant defense system.

ETHICAL APPROVAL

Experimental protocols used in this study were approved by the Ethical Committee of the Department of Animal Science of the University of Dschang-Cameroon (ECDAS-Uds 26/07/2017/UDs/FASA/DSAES) and were in conformity with the internationally accepted standard ethical guidelines for laboratory animal like described in the European Community guidelines; EEC Directive 86/609/EEC, of the 24th November 1986.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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